UPN "VETERAN" JATIM

STIKOM BALI

PROCEEDINGS

BALI INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY (BISSTECH) II 2014

"Fundamental and Applied Research for Industrial Sustainability: Food, Agrochemical, and Information and Communication Technology (ICT)"

September 2 - 4, 2014
BALI - INDONESIA

FACULTY OF INDUSTRIAL TECHNOLOGY - UPN "VETERAN" JAWA TIMUR
STIKOM BALI

ISBN: 978-602-9372-70-0

ARTICLES OF BALI INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY (BISSTECH) II 2014
"Fundamental and Applied Research for Industrial Sustainability: Food, Agrochemical, and Information and Communication Technology (ICT)"
September 2-4, 2014, BALI-INDONESIA

IMMUNOSTIMULATORY AND PREBIOTIC ACTIVITIES OF INULIN EXTRACTED FROM LESSER YAM TUBER (*DIOSCOREA ESCULENTA*)

Sri Winarti[1)], Eni Harmayani[2)], Yustinus Marsono[2)], Yudi Pranoto[2)], Kosuke Nishi[3)] and Takuya Sugahara[3)]
[1)] Food Technology Program, Faculty of Industrial Technology, Pembangunan National University of "Veteran" East Java, Surabaya, 60294, Indonesia
E-mail : swin_tpupn@yahoo.com
[2)] Food and Science Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, Indonesia.
[3)] Faculty of Agriculture, Ehime University, Matsuyama, Ehime 790-8566, Japan

Abstract

Inulin was extracted from lesser yam tuber (Dioscorea esculenta) in hot water. The objective of this study was to evaluate the effect of inulin from lesser yam tuber on the immune system by stimulatory production of IL-6 and TNF-α, and the effect of inulin as prebiotics. IL-6 and TNF-α produced by murine macrophage-like cell line J774.1 cells and murine primary peritoneal macrophages (P-Mac) were determined by ELISA. Prebiotic activity assay in vitro was performed by using Bifidobacteria and Lactobacilli. Profile and levels of SCFA (short chain fatty acid) were determined by GC (gas chromatography). Inulin from lesser yam tuber enhanced the production of IL-6 and TNF-α both by J774.1 cells and P-Mac at 8 μg/mL of inulin. This indicated that inulin from lesser yam tuber can be used as the immunostimulatory functional foodstuff. Prebiotic activity score of inulin extracted from lesser yam tuber was 1.21 on Lactobacillus casei FNCC-90 at 72 h, whereas commercial inulin was 0.90. Inulin from lesser yam tuber was increased the SCFA production by Bifidobacteria and Lactobacilli. This indicated that inulin extracted from lesser yam tuber may be used as prebiotics.

Keywords: inulin, *Dioscorea esculenta*, macrophage, cytokine, prebiotic, SCFA

1. Introduction

Inulin is dietary fiber chemically composed of a mixture of oligo- and/or polysaccharides constituted by fructose unit chains (linked by β-(2,1)-D-fructosyl-fructose bonds) with various lengths, terminated by a single glucose unit (linked by an α-D-glucopyranosyl bond) (French, 1993; Roberfroid & Delzenne, 1998). Inulin is a group of fructan β-(2-1) in which almost all linear chains of fructose have GFn structure (with G= unit glucosyl, F=fructosyl unit, and n=number of units of the chain fructosyl each other). For example, inulin extracted from chicory has the chemical structure of α-D-Glu-(1-2) - [(β-D-Fru-(1-2)-]n as a polymer molecule with a length between DP (degrees of polymerization) 3 to 60 (Roberfroid, 2005).

Inulin cannot be digested by enzymes present in the digestive tract of mammals but can be selectively fermented by colonic bacteria, so it is positively affect on the health of its host. Several types of *Bifidobacteria* can utilize inulin as an energy source by producing extracellular inulinase that hydrolyzes the β-(2-1)-D-fructose-fructosyl bond to fructose (Robertfroid, 2005). Inulin and oligofructose (OF) are classified as prebiotics.

Prebiotics are the food components that cannot be digested and can selectively stimulate the growth and the activity of beneficial bacteria in the digestive tract, specifically bifidobacteria and lactobacilli (Gibson, 2004; Pompei et al., 2008; Gaggia, et al., 2010). In the large intestine, prebiotic ingredients are fermented by probiotic bacteria, especially *Bifidobacteria* and *Lactobacilli*, and produce short chain fatty acids (SCFA), such as acetic, propionic, butyric, and lactic acid. SCFA can be used as an energy source by microorganisms.

The use of prebiotics such as oligosaccharides has been considered as a means of influencing the gut microbiota and risk of allergy. Non-digestible carbohydrates such as inulin and OF, including their intestinal fermentation products, may modulate the gut-associated lymphoid tissue (GALT) as well as the systemic immune system. The gut is a complex environment influenced largely by the microbial contents, bacterial secondary metabolites, immune-modulators and quorum-sensing molecules, and host factors including secretions (Louis et al. 2007). Simple molecules such as mono-, di- and oligosaccharides derived from complex carbohydrates during digestion, may exert these biological effects. The

ISBN: 978-602-9372-70-0

complex carbohydrates exert an effect on the gut-associated immunity before absorption, which is then transferred to the systemic immune responses via lymph nodes and Peyer's patches. Studies on the immunostimulatory effect of carbohydrates, especially oligosaccharides including inulin in humans, are very important.

Inulin is widely used in food industry in Europe, USA, Canada and Indonesia as a component (ingredient) in varrious of food products. It is naturally found as plant storage carbohydrates in Jerusalem artichoke (*Helianthus tuberosus*) (Lingyun et al., 2007), roots of *Morinda officinalis* (Yang et al., 2011), *Agave tequilana* (Arrizon et al., 2010), dahlia tubers, bananas and wheat (Robertfroid, 2005), commercial inulin produced from *Cichorium intybus* tubers (Toneli et al., 2008).

Dioscorea esculenta is one of the many types of *Dioscorea spp.* growing in Indonesia and its tuber contains the highest amount of inulin (14,77% db) (Winarti et.al, 2011). The tuber is very important as an alternative source of carbohydrate in the countryside. Isolation and characterization of inulin from *D. esculenta* tuber (lesser yam) have been performed, we have found that the yield of inulin was 21.33%, purity 73.585%, average solubility 76.77%, water content 13.68% and DP was 6 (Winarti et al., 2013). However, information on the immunostimulatory and prebiotic effects of inulin from lesser yam tuber is limited. The objective of this research was to evaluate the immunostimulatory and prebiotic activities *in vitro* of the inulin extracted from lesser yam tuber.

2. Materials and Methods

2.1. Prepreration of inulin

The isolation of inulin followed the method of Park et al. (2006) and Toneli, et al. (2008). Lesser yam tubers were washed, peeled and cut into small pieces, then blended with the addition of hot water at 80-90°C at the ratio of 1:20 (tuber:water). Slurry was performed in a shaking water bath at 90°C for 1 hour, filtered, cooled, and then frozen at -20 °C for 24 hours. The frozen filtrate was thawed and then centrifuged at 15,000g, for 15 minutes. White precipitate was dried at 60°C, for 5 hours, ground into powder and sieved. Inulin solution was prepared by suspending the inulin powder in hot distilled water at 2g/50 mL. The suspension was heated at 121°C for 20 min. The insoluble substances were removed by centrifugation at 15,600 x *g* for 20 min. The supernatant was filtered by millex GV filter 0.22 µm (Millipore, Billerica, MA). The commercial inulin (Februline Instant, native chicory inulin) produced by Cosucra Warcoing Group SA, Belgium (inulin SD) was used as a standard.

2.2. Culture of J774.1 cells

Murine macrophage-like cell line J774.1 cells were obtained from Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). J774.1 cells have been used in the screening of immune-stimulating activity for natural products (Hwang et al., 2010). J774.1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air for 24 h. Before observing the stimulating activity or harvesting the cells, the adherent cells were gently detached with phosphate buffered saline (PBS) containing 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA).

2.3. Preparation of mouse primary peritoneal macrophages (P-Mac)

Two milliliters of 4.05% thioglycolate medium were injected to the peritoneal cavity of BALB/c mice and left for 3 days to deteriorate the peritoneum. Resident P-Mac was harvested by washing the peritoneal cavities with 4 mL of 0.05% EDTA-PBS. After 5 min, the intraperitoneal fluid was collected using a syringe and centrifuged at 1200 x *g* for 5 min. The cell pellet was washed with PBS and centrifuged again. The cell pellet was suspended in 10% FBS-RPMI 1640 medium and cultured in a culture dish. After 24 h, unattached cells such as neutrophils were removed by aspirating the culture medium. All animal experiments described herein were carried out in accordance with protocols approved by the Ehime University Animal Care and Use Committee and were performed in accordance with applicable guidelines and regulations for the Care and Use of Laboratory Animals of Ehime University.

2.4. Effect of inulin on IL-6 and Tumor necrosis factor-α (TNF-α) production

J774.1 cells were cultured at 4.5×10^5 cells/mL and P-Mac at 5.2×10^5 cells/mL in 10% FBS-RPMI 1640 medium for 24 h. After 24 h, the medium was changed to the RPMI 1640 medium supplemented with 10 µg/mL of insulin, 20 µg/mL of transferrin, 20 µM ethanolamine, and 25 nM sodium selenite (ITES-RPMI 1640 medium) supplemented with lesser yam inulin or commercial inulin. After cultivation, the amounts of IL-6 and TNF-α in each culture medium were determined by ELISA kits (eBiosource, San Diego, CA, USA)

ISBN: 978-602-9372-70-0

according to the manufacture's instruction. Cytokine production assays were conducted in triplicate.

2.5. Bacterial cultures storage

Five strains of bacteria used in this research were *Bifidobacterium bifidum* BRL-130, *Bifidobacterium breve* BRL-131, *Bifidobacterium longum* ATCC-15707, *Lactobacillus casei* FNCC-90 and *Escherichia coli* FNCC-0051 from Food Nutrition and Culture Collection, Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, in the form of ampoules.These bacteria were cultured in the MRS broth medium (for *Bifidobacteria* and *Lactobacilli*) and TS broth (for *E. coli*). After a 24 h culture, 1 mL of each culture medium was collected in a sterilized micro tube, and then centrifuged at 3,000 x *g* for 15 min. After the medium was removed, 1 mL of a mixture of 10% skim milk and 20% sterile glycerol was added to the sediment cells, vortexed and stored at -20°C.

2.6. Preparation of growth media

The growth media used for this research were prepared by replacing glucose with inulin. MRS was used for prebiotic activity test and bacterial growth of *Bifidobacteria* and *Lactobacilli*. Tryptic Soy agar and broth made from: 15 g of tryptone, 5 g of soya peptone, 5 g of sodium chlorite with and without 15 g of agar, dissolved in one liter of water. These media were used for the preparation, reculture and calculations of *E.coli*. M-9 medium made from: 64 g of $Na_2HPO_4.7H_2O$, 15 g of KH_2PO_4, 2.5 g of NaCl, 5.0 g of NH_4Cl, dissolved in one liter of water and then sterilized by autoclave at 121 °C, for 15 min. For prebiotic activity test of *E. coli*, 200 mL of M-9 solution was added with 700 mL sterile distilled water, 2 mL of 1 M $MgSO_4$, 20 mL of 20% glucose or other carbon sources, 100 µL of 1 M $CaCl_2$ up to 1000 mL. The medium was used for testing growth of *E. coli* with glucose and inulin as an energy source.

2.7. Prebiotic activity assay

Bifidobacterium bifidum BRL-130, *Bifidobacterium breve* BRL-131, *Bifidobacterium longum* ATCC-15707 and *Lactobacillus casei* FNCC-90 were grown in MRS broth and *Escherichia coli* FNCC-0051 in TS broth for 24 h, with glucose or inulin as carbon source. These bacteria were then diluted with sterile physiological saline at the cell number of approximately 10^5cells/mL. This culture was inoculated in the treating medium and incubated for 0, 24, 48 and 72 h. The total bacteria were enumerated by the total plate count (TPC) method.

2.8. Determination of prebiotic activity score (Huebner, *et al.*, 2007)

Prebiotics Activity Score:

$$\text{Prebiotic activity score} = \left\{\frac{(\text{probiotic log cfu/ml on the prebiotic at 24 h} - \text{probiotic log cfu/ml on the prebiotic at 0 h})}{(\text{probiotic log cfu/ml on the glucose at 24 h} - \text{probiotic log cfu/ml on the glucose at 0 h})}\right\} - \left\{\frac{(\text{enteric log cfu/ml on the prebiotic at 24 h} - \text{enteric log cfu/ml on the prebiotic at 0 h})}{(\text{enteric log cfu/ml on the glucose at 24 h} - \text{enteric log cfu/ml on the glucose at 0 h})}\right\}$$

2.9. Analysis of SCFA (acetate, propyonate and butyrate)

Profiles and levels of acetic, propionic and butyric acid during fermentation were analyzed at 72 hours using a Shimadzu GC 8A with a column of GP 10% SP 1200/1% H_3PO_4 on 80/100 Cromosob WAW in a 3 mm diameter, 2 m long column and FID detector type with test conditions at 140°C column temperature, 240°C detector temperature, carrier gas N_2, 1.5 kg/cm^2 pressure and sample injected 1 µL.

3. RESULTS AND DISCUSSION

3.1. Effect of inulin on cytokine production by J774.1 cells

Cytokines are signaling molecules that control homeostasis of the immune system by the regulation of cell differentiation, proliferation and apoptosis, as well as defense functions such as immune responses and inflammatory reactions. Among the proinflammatory cytokines, IL-6 is one of the most important mediators of fever and the acute-phase response (Gabay, 2006). TNF-α also plays an important role as a key cytokine in immune and inflammatory reactions. TNF-α has direct *in vitro* and *in vivo* cytostatic and cytocidal effects. In addition, together with IL-6, TNF-α is also considered as a major inflammatory mediator (Gabay, 2006). One of the most prominent characteristics of TNF-α is its ability to cause apoptosis of tumor-associated endothelial cells, resulting in tumor necrosis (Lejeune et al., 2006). TNF-α also plays a pivotal role in host defense and can act on monocytes and macrophages in an

ISBN: 978-602-9372-70-0

ARTICLES OF BALI INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY (BISSTECH) II 2014
"Fundamental and Applied Research for Industrial Sustainability: Food, Agrochemical, and Information and Communication Technology (ICT)"
September 2-4, 2014, BALI-INDONESIA

autocrine manner to enhance various function responses and induce the expression of a number of other immunoregulatory and inflammatory mediators (Baugh and Bucala, 2001). Many polysaccharides can activate macrophages by inducing the production of cytokines (Belska et al., 2010; Lee et al., 2010; Togola et al., 2008). Our study demonstrated that the lesser yam inulin and commercial inulin effectively enhanced IL-6 (Fig 1) and TNF-α production (Fig 2) by J774.1 cells. Inulin stimulated the production of IL-6 and TNF-α in dose- and time- dependent manners.

As shown in Fig. 1, lesser yam inulin stimulated the IL-6 production by J774.1 cells about 4.4-fold at 6 h at 8 μg/mL, whereas commercial inulin about 2.3-fold at 6 h at 24 μg/mL compared with control. The IL-6 production stimulatory activity of inulin was higher than that of LPS (lipopolysaccharides) at 100 ng/mL (from *E.coli* 026:B6, Sigma).

LPS is a component of the cell wall of Gram-negative bacteria. LPS and its lipid A moiety stimulate cells of the innate immune system through the Toll-like receptor 4 (TLR4), a member of the Toll-like receptor protein family, which recognizes common pathogen-associated molecular-patterns (PAMPs).

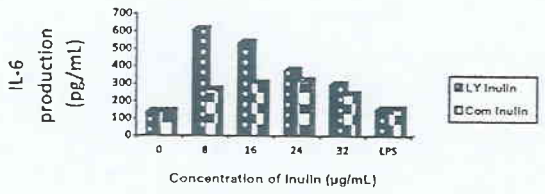

Fig. 1. Effects of lesser yam inulin (LY inulin) and Comercial inulin (Com inulin) on IL-6 production by J774.1 cells. J774.1 cells were inoculated at $4.5x10^5$ cells/mL and incubated for 6 h.

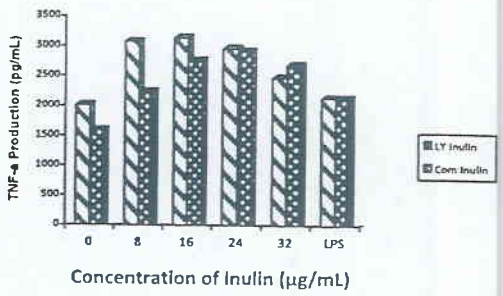

Fig. 2. Effects of lesser yam inulin (LY inulin) and Commercial inulin (Com inulin) on TNF-α production by J774.1 cells. J774.1 cells were inoculated at $4.5x10^5$ cells/mL and incubated for 6 h.

As shown in Fig. 2, lesser yam inulin stimulated the TNF-α production by J774.1 cells about 1.6-fold at 6 h at 8 μg/mL, whereas commercial inulin about 1.9-fold at 6 h at 24 μg/mL against control. The activity of inulin was higher than that of LPS from *E.coli* 026:B6 (Sigma) at 100 ng/mL.

The activation of macrophages is a key event in the innate immunity for initiating and propagating defensive reactions against pathogens (Mishra et al., 2006). Activated macrophages induce the productions of nitric oxide (NO) and inflamatory cytokines such as TNF-α and IL-6. TNF-α is mainly produced by activated macrophages, T lymphocytes, and natural killer (NK) cells. The roles of TNF-α include activation and chemotaxis of leukocytes and induction of the expression of adhesion molecules, such as intercellular adhesion molecule-1, on neutrophils and endothelial cells (Jiang et al., 2005).

3.2. Effect of inulin on cytokine production by P-Mac

Although various food components especially polyshaccharides have been screened by J774.1 cells, there was no report concerning the screening of the immunological effects of inulin on primary peritoneal macrophages (P-Mac). In this study, the immunostimulatory activities of inulin extracted from lesser yam tubers have been screened using P-Mac. The data revealed that the lesser yam inulin stimulated production of IL-6 (Fig 3) and TNF-α (Fig 4) by P-Mac.

ISBN: 978-602-9372-70-0

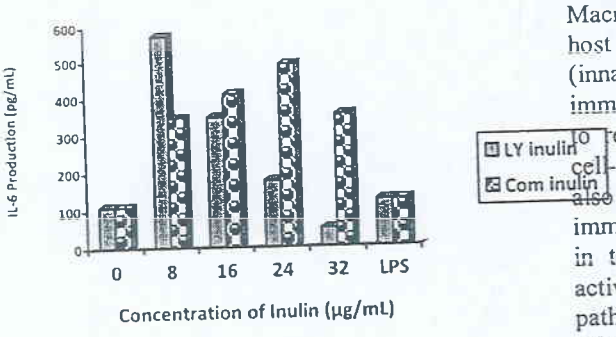

Fig. 3. Effects of lesser yam inulin (LY inulin) and Commercial inulin (Com inulin) on IL-6 production by P-Mac. P-Mac were inoculated at $5.2x10^5$ cells/mL and incubated for 6 h.

As shown in Fig. 3, lesser yam inulin stimulated the IL-6 production by P-Mac about 5.17-fold at 6 h at 8 µg/mL, whereas commercial inulin about 4.5-fold at 6 h at 24 µg/mL against control. The activities of inulins were higher than that of 100 ng/mL of LPS. In Fig. 4, lesser yam inulin stimulated the TNF-α production by P-Mac about 2.1-fold at 6 h at 8 µg/mL, whereas commercial inulin about 2.3-fold at 6 h at 24 µg/mL against control. The activities of inulins were higher than that of 100 ng/mL of LPS.

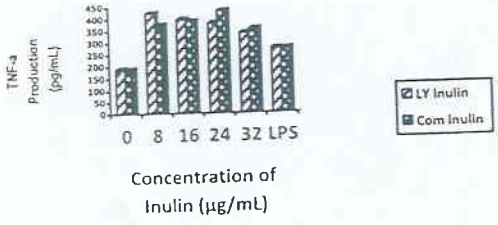

Fig. 4. Effects of lesser yam inulin (LY inulin) and Commersial inulin (Com inulin) on TNF-α production by P-Mac. P-Mac were inoculated at $5.2x10^5$ cells/mL and incubated for 6 h.

Both lesser yam inulin and commercial inulin were stimutated production of cytokine by P-Mac. Activated macrophages produce NO and cytokines. NO is an important intra- and inter-cellular regulatory molecule with multiple biological functions, including macrophage-mediated cytotoxicity (Moncada et al., 1991). Macrophages and lymphocytes play a major role in host defense as part of the non-specific defense (innate immunity) and specific defense (adaptive immunity) systems. Macrophages are the first cells to recognize infectious agents and are central to cell- mediated and humoral immunity. TNF-α is also a significant regulator of the inflammatory and immune responses. These molecules are involved in the nuclear factor-kB (NF-kB) and mitogen-activated protein kinase (MAPK) signaling pathways. The majority of the spleen's functions is related to the immune system. The spleen stores and produces lymphocytes that produce antibodies and assist in removing microbes and other debris from the blood (Brown et al., 2002). Lymphocytes are a type of white blood cell in the vertebrate immune system and can be divided into NK cells, T cells, and B cells. NK cells are part of the innate immune system and play a major role defending the host from both tumors and virally infected cells. T and B cells are the major cellular components of the adaptive immune response (Vivier et al., 2011).

3.3. Prebiotic activity score of inulin from lesser yam tuber

The prebiotic activity score of inulin extracted from lesser yam tuber and of commercial inulin are presented in Fig 5. The highest prebiotic activity score of inulin from lesser yam tuber was 1.21 by *Lactobacillus casei* FNCC-90 followed by *Bifidobacterium breve* BRL-131 was 1.17 at 72 h. The highest prebiotic activity score of commercial inulin was 0.902 by *Lactobacillus casei* FNCC-90, followed by *Bifidobacterium breve* BRL-131 (0.753) at 72.

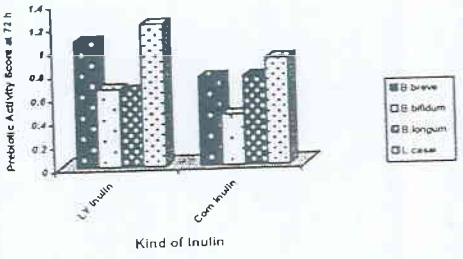

Fig 5. Prebiotic activity score lesser yam inulin and commercial inulin

Differences in the prebiotic activity score were caused by difference in ability on the metabolism of prebiotic compounds on each bacterial strain. Differences in the metabolic

ISBN: 978-602-9372-70-0

capability on prebiotic compounds require the presence of transport systems and specific hydrolysis for prebiotic. Gene that encodes a specific metabolic system may be present or not on each *Bifidobacteria* and *Lactobacillus* strain (Huebner, *et al.*, 2007).

Huebner *et al.* (2007) showed that the prebiotic activity score of Inulin-S, Raftiline HP, Raftilose P95 by *Lactobacillus paracasei* 1195 were 1.17, 1.10 and 0.99 respectively; by *Lactobacillus plantarum* 4008 was 0.82 on GOS, *Lactobacillus acidophilus* 33200 was 0.70 on GOS, *Lactobacillus acidophilus* NCFM was 0.66 on GOS and *Lactobacillus acidophilus* NCFM was 0.58 on Raftilose P95. Prebiotic activity scores of *B.bifidum* NCI grown on GOS and Inulin-S were negative (-1.24 and -1.17).

3.4. Profile of SCFA (acetate, propionate and butyrate)

Acetic acid formation by *Bifidobacteria* and *Lactobacilli* in media containing inulin from lesser yam tuber, glucose and commercial inulin as an energy source at 72 h is presented in Fig. 6. The highest acetic acid was produced by *Bifidobacterium longum* ATCC-15707 (113.79 mmol) in medium containing lesser yam inulin, followed by *Bifidobacterium bifidum* BRL-131 (100.81 mmol).

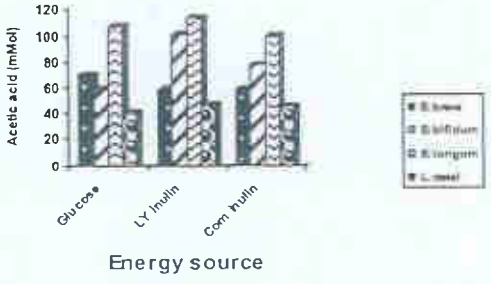

Fig.6. Profile of acetic acid produced by *Bifidobacteria* and *Lactobacilli* in the medium containing glucose, lesser yam inulin and commercial inulin as a carbon source

Propionic acid formation by *Bifidobacteria, Lactobacillii* and *E. coli* with the medium containing inulin extracted from lesser yam tuber, glucose and commercial inulin as an energy sources at 72 h is presented in Fig 7. The highest acetic acid was produced by *Bifidobacterium longum* ATCC-15707 was 19.217 mmol in medium containing inulin from lesser yam tuber, followed in glucose was 14.229 mmol and in commercial inulin was 12.057 mmol.

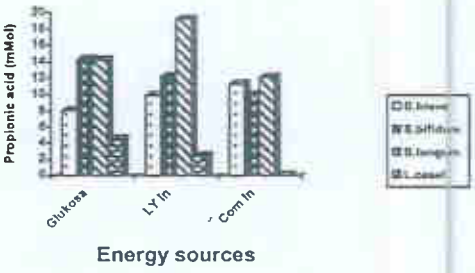

Fig. 7. Profile of propionic acid formation by *Bifidobacteria* and *Lactobacilli* in medium glucose, inulin from lesser yam tuber and commercial inulin as an energy source

The profile of butyric acid formation on fermentation by *Bifidobacteria* and *Lactobacilli* with the medium containing inulin from lesser yam tuber, glucose and commercial inulin as an energy source at 72 h is presented in Fig. 8.

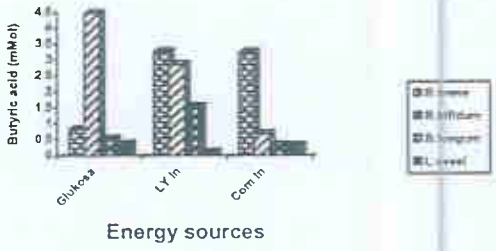

Fig. 8. Profile of butyric acid formation by *Bifidobacteria* and *Lactobacilli* in medium glucose, lesser yam inulin and commercial inulin as an energy source

The highest of butyric acid was produced by *Bifidobacterium bifidum* FNCC-131 in the medium containing glucose, followed by *Bifidobacterium breve* FNCC-131 in the medium containing lesser yam inulin was 3.262 mmol, and in the medium containing commercial inulin was 3.252 mmol. The formation of SCFA during fermentation in the medium containing lesser yam inulin was higher than in the commercial inulin. Acetic and propionic acid produced by *Bifidobacterium longum* ATCC-15707, while butyric acid by *Bifidobacterium breve* FNCC-130.

This indicated that each polysacharide was hydrolyzed at different rates by different microbes. Inulin is polyfructose which is metabolized by intestinal microbiota, including *Bifidobacteria* through the glycolytic pathway to produce pyruvate, then pyruvate is converted to Acetyl-co-A, lactate and succinate. Acetil-co-A can be converted to acetate, butyrate, while succinate is converted to propionate and formiate is converted to gas H_2, CH_4 and/or H_2S (Macfarlane and Macfarlane, 2003). Hexoses used by *Bifidobacteria* through an unusual metabolic pathway called "bifid shunt" (Robertfroid, 2005; Tannock, 2010). *Bifidobacteria* genus has a unique metabolic pathway that produces the enzyme fructose-6-phosphate phosphoketolase to ferment oligosaccharides. The enzyme is a key enzyme to recognize the genus (Sela *et al.*, 2010).

4. CONCLUSIONS

Inulin extracted from lesser yam tuber enhanced the production of IL-6 and TNF-□ by J774.1 cells and P-Mac. This indicated that inulin from lesser yam tuber can be used as immunostimulatory. Inulin extracted from lasser yam tuber had the higher prebiotic activity score than the commercial inulin. Inulin extracted from lesser yam tuber also enhanced the production of SCFA during fermentation. This indicated that inulin from lesser yam tuber can be used as prebiotic.

5. ACKNOWLEDGEMENTS

Authors thank to Directorate of Human Resources Directorate General of Higher Education Ministry of National Education via. Sandwich-like Program 2013 was financial supported in this research.

6. Reference

[1] Akin, M.B., M.S. Akin and Z. Kirmaci, 2007. Effect of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chemistry. 104,93-99.

[2] Arrizon, J., S. Morel, A. Gschaedler, and P. Monsan, 2010. Comparison of the water-soluble carbohydrate composition and fructan structures of Agave teguilana plants of different ages. Food Chemistry 122:123-130.

[3] Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D.L., Martinez-Pomares, L., Wong, S. Y., & Gordon, S. (2002). Dectin-1 is a major b-glucan receptor on macrophages. The Journal of Experimental Medicine, 196, 407–412.

[4] Crittenden, R.G & M.J.Playne. 1999. Production, Properties and Applications of Food-grade Oligosaccharides. Trends in Food Science and Technology 7, 353-361.

[5] Gabay, C., (2006). Interleukin-6 and chronic inflammation. Arthritis Research&Therapy, 8(Suppl. 2),S3.

[6] Gaggia, F., P. Mattarelli and B. Biavati, 2010. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology 141(2010)515-528.

[7] Gibson, G.R., Beatty, E.R., Wang X., Cummings J.H.,1995. Selective stimulation of Bifidobacteria in human colon by oligofructosa and inulin. Gastroenterology; 108:975-982.

[8] Gibson, G.R., 2004. Prebiotics. Best Practice & Research Clinical Gastroenterology. Vol.18, No.2, pp.287-298.

[9] Huebner, J., R.L.Wehling, and R.W. Hutkins. 2007. Functional activity of commercial prebiotics. International Dairy Journal. 17(2007) 770-775.

[10] Hwang, P. A., Wu, C. H., Gau, S. Y., Chien, S. Y., & Hwang, D. F. (2010). Antioxidant and immune-stimulating activities of hotwater extract from seaweed Sargassum hemiphyllum. Journal of Marine Science and Technology, 18, 41–46.

[11] Jiang, M.Z., Tsukahara, H., Hayakawa, K., Todoroki, Y., Tamura, S., Ohshima, Y., Hiraoka, M., Mayumi, M., 2005. Effects of antioxidants and NO on TNF-alphainduced adhesion molecule expression in human pulmonary microvascular endothelial cells. Respir. Med. 99, 580–591.

[12] Kaur, A., D.J. Rose, P. Rumpangaporn, J.A. Peterson and B.R. Hamaker. 20011. In vitro batch fecal fermentation comparison of gas and Short-Chain Fatty Acid production using "slowly fermentable" dietary fibers. Journal of Food Science. Vol.4, (2).211-218.

[13] Lee, J.S., Kwon, J.S., Yun, J.S., Pahk, J.W., Shin, W.C., Lee, S.Y., Hong, E.K., 2010. Structural characterization of immunostimulating polysaccharide from cultured mycelia of Cordyceps militaris. Carbohydr. Polym. 80, 1011–1017.

[14] Lingyun, W., W. Jianhua, Z. Xiaodong, T. Da, Y. Yalin, C. Chenggang, F. Tianhua, and Z. Fan. 2011. Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers. Journal of Food Engineering 79:1087-1093.

[15] Louis, P., K.P.Scott, S.H. Duncan dan H.J.Flint. (2007). Understanding the effect of diet on bacterial metabolism in the large intestine. Journal of Applied Microbiology, 102:1197-1208 (Review Article)

[16] Macfarlane, S. and G.T. Macfarlane, 2003. Regulation of short-chain fatty acid production. Proceedings of the Nutrition Society (2003),62,67-72.

[17] Mishra, K.P., Padwad, Y.S., Jain, M., Karan, D., Ganju, L., Sawhney, R.C., 2006. Aqueous extract of Rhodiola imbricata rhizome stimulates proinflammatory mediators via phosphorylated IkappaB and transcription factor nuclear factor-kappaB. Immunopharmacol. Immunotoxicol. 28, 201–212.

[18] Moncada, S., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

[19] Oliviera, R.P.D.S, A.C.R.Florence, P.Perego, M.N.D.Oliviera and A.Converti, 2009. Use of lactulosa as prebiotic its influence on the growth, acidification profile and viable count of different probiotics in fermented skim milk. International Journal of Food Microbiology. 145(2011)22-27.

[20] Oliviera, R.P.D.S, P.Perego, M.N.D.Oliviera and A.Converti, 2011. Effect of inulin as a prebiotic to improve growth and count of a probiotic cocktail in fermented skim milk. LWT-Food Science and Technol. 44(2011)520-523.

[21] Park, K.J., Toneli, J.T.C.L., Elisabeth, F., Martinelli, P., 2006. Optimazion of Physical Concentration Process for Inulin. School of Food Engineering State University of Campinas (UNICAMP): Brazil.

[22] Pompei, A.,L.Cordisco, S. Raimondi, A. Amaretti, dan U.M. Pagnoni, 2008. In vitro comparation of the prebiotic effect of two inulin-type fruktans. Aerobe 14(2009),280-286.

[23] Putra, A.B.N., Morishige, H., Nishimoto, S., Nishi, K., Shiraishi, R., Doi, M., & Sugahara, T. (2012). Effect of collagens from jellyfish and bovine Ahilles tendon on the activity of J774.1 and mouse peritoneal macrophage cells. Journal of Functional Foods,4, 504–512.

[24] Roberfroid, M.B., 2005. Introducing inulin-type fructans. British Journal of Nutrition, 93, Suppl.1,S13-S25.

[25] Rogge, T.M., 2005. Chemical Modification of Inulin. Synbioc, Faculty of Bioscience Engineering, Universitet Gent.

[26] Sela, D.A., P.J. Price and D.A. Mills. 2010. Metabolism of bifidobacteria. In : Bifidobacteria : Genomic and Molecolar Aspects. Caister Academic Press.

[27] Sun, Y., Liu, J., 2008. Structural characterization of a water-soluble polysaccharide from the roots of Codonopsis pilosula and its immunity activity. Int. J. Biol.

[28] Tannock, G.W. 2010. Analysis of bifidobacterial populations in bowel ecology studies. In : Bifidobacteria : Genomic and Molecolar Aspects. Caister Academic Press.

[29] Toneli, J.T.C.L., K.J. Park, J.R.P. Ramalho, F.E.X. Murr dan I.M.D. Fabbro, 2008. Rheological Characterization of Chicory Root (*Cichorium intybus* L.) Inulin Solution. *Brazilian Journal of Chemical Engineering,* Vol.25, No.03, 461-471.

[30] Vivier, E., Raulet, D.H., Moretta, A., Caligiuri, M.A., Zitvogel, L., Lanier, L.L., Yokoyama, W.M., Ugolini, S., 2011. Innate or adaptive immunity? The example of natural killer cells. Science 331, 44–49.

[31] Wang, Y.2009. Prebiotics: Present and future in food science and technology. Review. Food Research International, 42(2009)8-12.

[32] Wang, J., Luo, J.,Yang, X., Zha, X., 2010. Structural analysis of a rhamnoarabinogalactan from the stems of Dendrobium nobile Lindl. Food Chem. 3, 572–576.

[33] Winarti, S., E., Harmayani and R., Nurismanto., 2011. Characteristics and inulin profile on varrious yam tubers (*Dioscorea spp.*). AGRITECH, Vol.31, No.4: 378-383.

[34] Winarti, S., E., Harmayani and Y., Marsono, and Y. Pranoto, 2013. Foaming effect on drying of lesser yam inulin (*Dioscorea esculenta*) on characteristic and prebiotic ctivities. AGRITECH, Vol.33 (4): 311-319.